



University of Nebraska Medical Center

Routine Urinalysis- Microscopic Examination of Urine

CLS 500: Application and Interpretation
of Clinical Laboratory Data



Objectives: Upon completion of this unit, the participant will:

- Describe routine UA microscopic exam
- Correlate common microscopic findings with possible clinical conditions
- Identify conditions that enhance urine cast formation
- Describe sources of error

2

Standardization of Microscopic Exam

- Strict adherence to laboratory protocol using the same:
 - Supplies
 - Sequence of procedural steps
 - Timing intervals
 - Equipment
- Ensures accuracy and precision of microscopic analysis of urine sediment

3

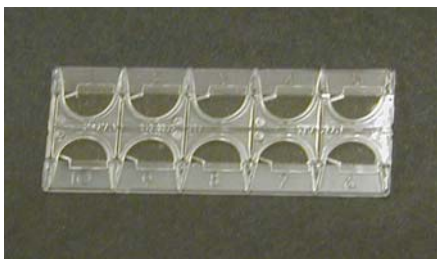
Commercial Systems

- Produce the same concentration of urine or sediment volume
- Present the same volume of sediment for microscopic analysis
- Control microscopic variables such as focal planes and optical properties of slides

4

Commercial Systems

KOVA slide system



5

7 Factors to Standardize

1. Specimen collection and handling
2. Specimen volume
3. Centrifugation
4. Sediment concentration
5. Volume of sediment examined
6. Consistent examination procedure
7. Reporting format and terminology

6



1. Proper Specimen

- Collection
 - Patient prep may or may not be done
 - Random, clean catch, catheterized, etc
- Rapid transport to lab
 - Room temperature, refrigerated specimen
- Prompt examination
 - Within 30 minutes of collection ideal



2. Volume of urine evaluated

- Well-mixed urine
- 12 milliliters (10-15 mL range) routine volume analyzed



8

3. Centrifugation

- Speed: 400-450 g
 - Relative centrifugal force (g)
 - Independent of centrifuge used and rotor size
 - Speed (RPM) required to attain 450g will vary in different centrifuges
 - Ensures optimal sediment concentration without disruption of fragile formed elements
- Time: 5 minutes
- No brake: will resuspend pellet of sediment

9

4. Urine Volume to resuspend Sediment

- 12:1 ratio desired:
 - Centrifuge 12 mL of urine
 - Decant urine using pipette, leaving 1 mL urine in bottom of test tube
 - Resuspend sediment in bottom of test tube with the 1 mL urine volume retained



10

5. Volume of resuspended sediment to examine

- Glass slides with coverslip: 15 microliters using calibrated pipette
- Commercial slide: viewing chamber fills by capillary action
- No bubbles, do not overfill

11

Commercial Systems

Example:
KOVA slide system



12



6. Consistent examination

- **Minimum** '10-12 representative fields'
- Elements must be evenly distributed throughout slide
- If not, prepare another wet prep
- What is a Field of View (FOV)?

13

Field of View (FOV)

FOV: what you see through the ocular lens



14

Low Power Objective

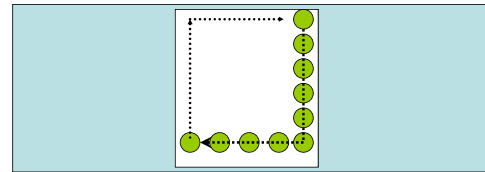
- 10x objective
- Examine perimeter of cover-slip
- Look for
 - Squamous epithelial cells
 - Casts
 - Mucus



15

Scanning the Microscope Slide

- Use 10X objective: scan the coverslip perimeter for squamous epithelial cells, cast and mucus
- Example: begin in the upper right hand corner, down the right side then the bottom, up and over
- Remember to scan a MINIMUM of 10 FIELDS



High Power Objective

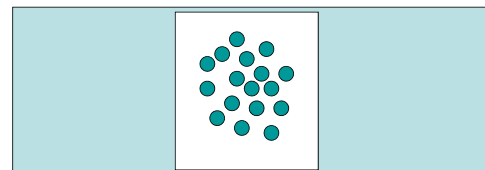
- 40x objective
- Examine center area of cover-slip
- Look for
 - RBC and WBC
 - Transitional and renal tubular epithelial cells
 - Bacteria
 - Yeast
 - Sperm
 - Trichomonads
 - Crystals



17

Scanning the Microscope Slide

- Use 40X objective, scan the interior of the slide
- Look for all other cellular elements
- Remember to scan a MINIMUM of 10 FIELDS





7. Report format and terminology

- Number per low power field:
 - 20-100 squamous epithelial cells/lpf
 - 0-3 hyaline casts/lpf
 - 5-10 granular casts/lpf
- Number per high power field:
 - 10-25 RBC/hpf
 - 5-10 renal tubular epithelial cells/hpf

19

Ensure Accuracy in Reporting

Microscopic results should be correlated to the physical and chemical results

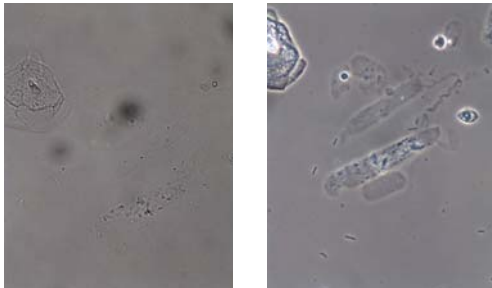
Microscopic Elements	Physical (color/clarity)	Chemical (dipstick)	Exceptions
Red blood cells	clarity, color	+ blood	Number, hemolysis
White blood cells	clarity	+ protein, leukocytes, nitrite	Number, type of WBC and bacteria
Epithelial cells	clarity		Number
Casts	clarity	+ protein	Number
Crystals	clarity, color	pH	Number, type
Bacteria	clarity	+ nitrite, pH	Number, type

If red blood cells are present, the color and clarity are affected (Red/Hazy), and the dipstick should show positive blood.

The exceptions are: if there are not enough RBCs or ascorbic acid is present (Red/Hazy, negative blood); or if the RBCs are lysed (Red/Clear, positive blood)

20

Microscopic Techniques

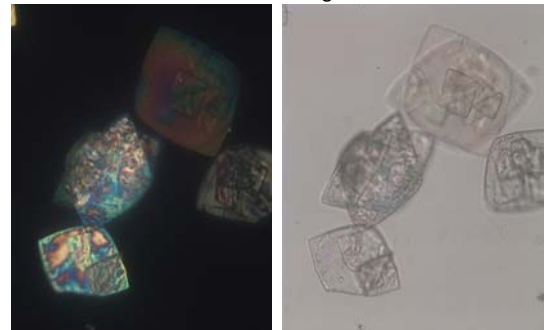


Brightfield vs Phase Contrast

21

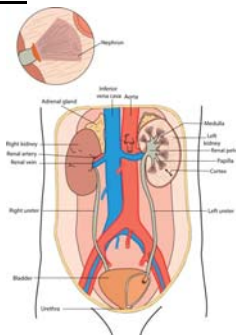
Microscopic Techniques

Polarization vs Brightfield



Formed Elements

- Originate throughout urinary tract
- Can be a result of damage to basement membrane, infection, disease
- Contaminant



23

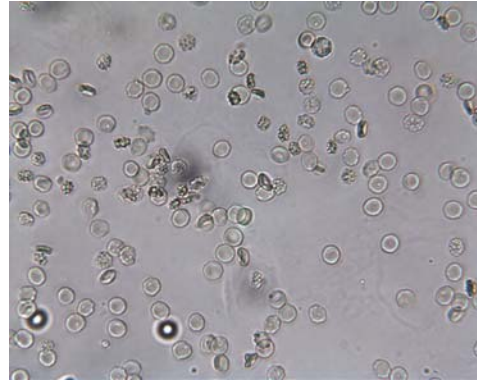
Red Blood Cells

- Intact RBC: hematuria
- Normal: 0-3 /hpf
- Abnormal: damage to basement membrane of glomerulus, kidney infection, kidney stones, trauma

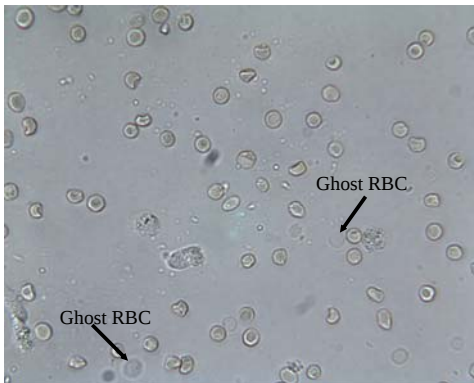
24



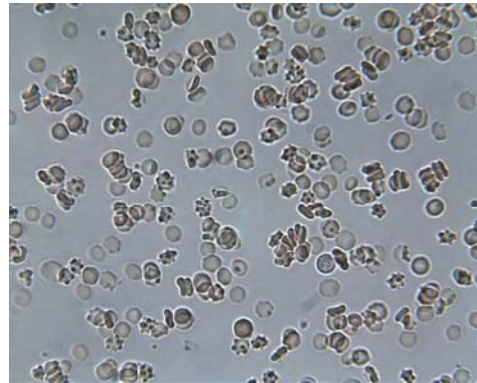
RBC: smooth biconcave discs, no nucleus 25



Concentrated urine: RBCs crenate 26



Dilute urine: RBCs will swell 27

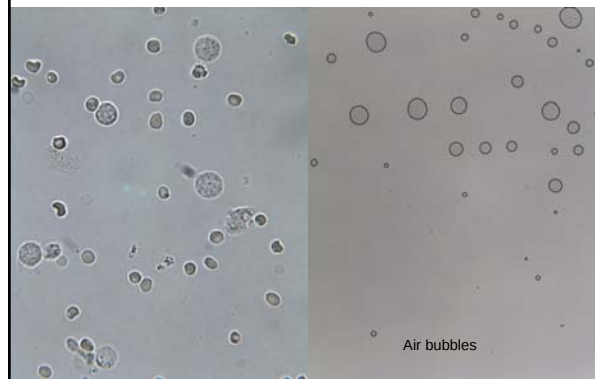


RBC's showing rouleaux 28

RBC can resemble:

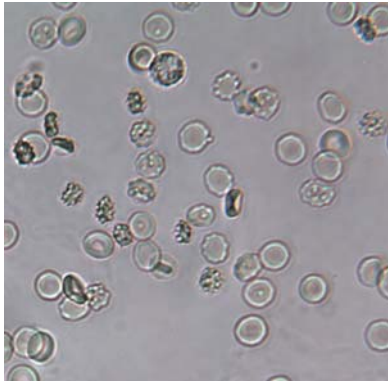
- Yeast
- Oil droplets, air bubbles
- Calcium oxalate crystals, oval form
- In concentrated urine, RBC will crenate and resemble WBC

29



Air bubbles

30



Crenated RBC can resemble WBC

31

RBC Correlation

- Correlate microscopic evaluation with
 - Physical exam
 - Color
 - Clarity
 - Chemical exam
 - Positive reagent strip
 - Ascorbic acid: causes false negative result
 - Myoglobin: causes false positive result

32

WBC: leukocytes

- WBC in urine: leukocyturia
- Indicates infection (bacterial, non-bacterial)
- Normal: 0-8 WBC/hpf
- Neutrophil predominant type of WBC found in urine

33

WBC: leukocytes

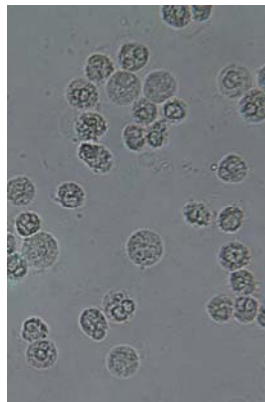
- Spherical
- Contain nucleus, granules, etc
- Approximately 2x larger than RBC



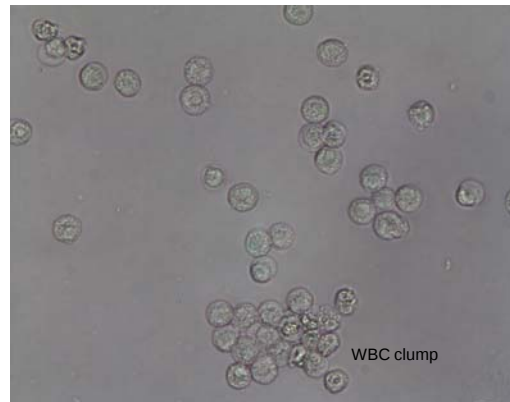
34

WBC: leukocytes

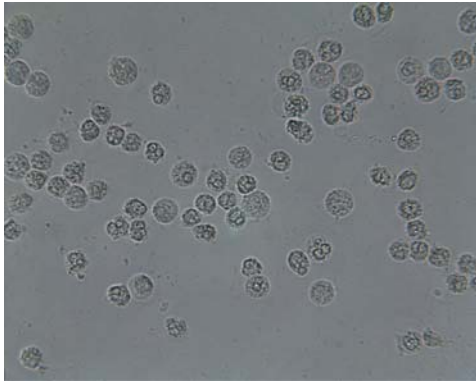
- Cytoplasm contains granules
- Nucleus is segmented (lobed)
- Can be found singly or in clumps



35



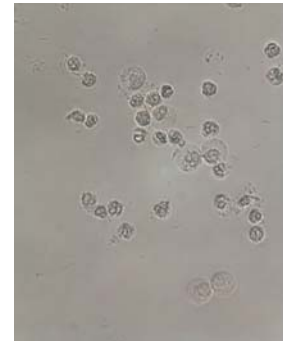
36



37

Hypotonic (dilute) Urine

- Dilute urine: WBC swell and then lyse
- Glitter cells: swollen WBCs showing brownian movement



Hypertonic (concentrated) Urine

- WBC become smaller, due to water moving out of the WBC
- Unlike RBC, WBC do not crenate



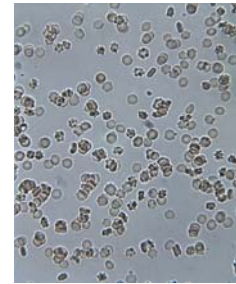
39

WBCs can resemble:

Renal tubular epithelial cells



Crenated RBC



40

WBC Correlation:

- Correlate microscopic evaluation with
 - Physical exam
 - Odor (infection)
 - Clarity
 - Chemical exam
 - Positive reagent strip for leukocyte esterase
 - Non-granular WBC (lymphocytes) will **not** react with reagent strip reaction (false negative)

41

Epithelial Cells

- Found in urine due to
 - Normal sloughing of old cells from lining of genitourinary system
 - Inflammation of the lining
 - Renal disease
 - Common contaminant in female specimens

42



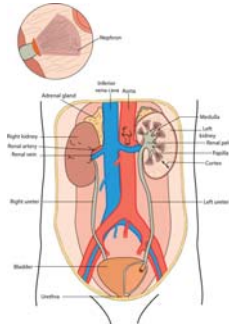
Epithelial Cells

- Three types:
 - Squamous epithelial cells
 - Transitional epithelial cells
 - Renal tubular epithelial cells
- Normal: small amount
- Abnormal: infections, disease

43

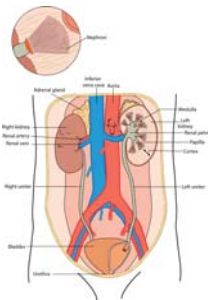
Squamous Epithelial Cells

- Most common type of epithelial cell found in urine
- Originates in superficial lining of urethra and vagina
- Increased numbers may indicate poor collection technique



Transitional Epithelial Cells

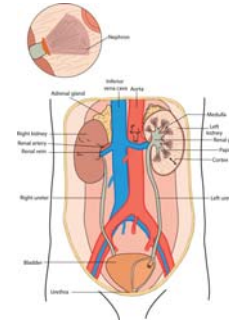
- Originates in lining of renal pelvis, ureters, BLADDER, upper urethra
- Increased numbers may indicate urinary tract infection (UTI), collection by catheterization, malignancy



45

Renal Tubular Epithelial Cells

- Originates in lining of RENAL TUBULES
- Increased numbers indicate tubular necrosis



46

Squamous Epithelial Cells

- Largest epithelial cell found in urine
- Enumerate using low power objective
- Cells are thin and flat; central nucleus
- Fine granulation in cytoplasm that becomes dense as cell degenerates



47

Clue Cells

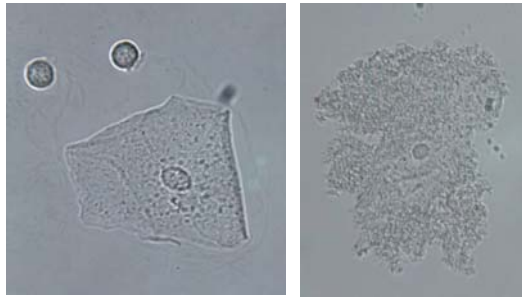
- Squamous epithelial cells with large amount of bacteria adhering to them giving them a 'shaggy' appearance
- Originates in vaginal mucosa, so considered vaginal contaminant; presence indicates bacterial vaginal infection



48



Squamous Epi vs Clue Cell



49

Transitional Epithelial Cells

- Size varies dependent upon location in urinary tract
- Most common type seen in urine originates in the bladder
- Generally much larger than WBC with abundant cytoplasm; **nucleus to cytoplasm ratio ~ 1:5**
- Nucleus generally centrally located
- Borders of nucleus and cytoplasm distinct

50

Transitional Cells



Evaluate and enumerate using high power objective

51

Renal Tubular Epithelial (RTE) Cells

- Shape varies dependent upon location in urinary tract
- Cells usually are round and slightly larger than WBC
- Nucleus is eccentric; can be multinucleated
- Nucleus to cytoplasm ratio ~ 1:1

52

RTE Cells



Evaluate and enumerate using high power objective
Notice the 'hard outline' of the nucleus

53

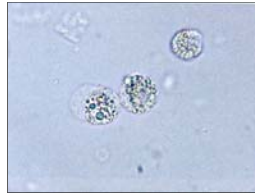
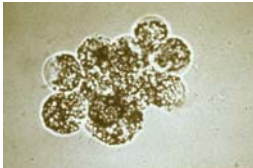
Oval Fat Bodies (OFB)

- Renal tubular epithelial cell with absorbed fat
- Highly refractile due to absorbed fat; amount of absorbed fat varies
- Laboratory must see maltese cross using polarized microscopy to verify cell is OFB
- May also see free floating fat droplets
- Indicates pathology: evaluate and enumerate using high power objective

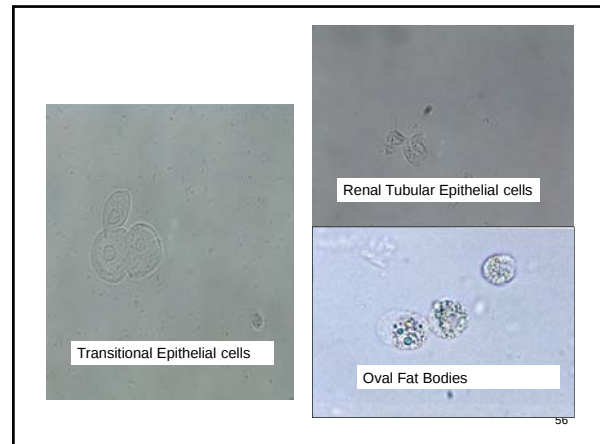
54



OFB



55



56

Epithelial Cell Correlation:

- Correlate microscopic evaluation with:
 - Physical exam
 - Clarity
 - Chemical exam:
 - Protein reagent strip reaction usually positive when RTE or OFB present

57

Type of Epithelial cell:

Characteristic:

- | | |
|---------------------|--|
| Squamous (lpf) | a. Most common epithelial cell found in urine |
| Transitional (hpf) | b. Originates in lining of renal tubules |
| Renal tubular (hpf) | c. Often seen in 'sheets' of cells from catheterized urine |
| Oval fat body (hpf) | d. Originates in superficial lining of urethra and vagina |
| | e. May indicate improper collection technique if increased numbers found |
| | f. Originates in bladder |
| | a. RTE with absorbed fat |

58

Casts

- Presence of casts reflect health status of renal tubules
 - Number and type of cast is significant
 - Increased amounts of casts: usually also have a positive protein dipstick
- Enumerated: lpf → Identified: hpf

59

Casts

- Normal: few hyaline or few granular casts
- Abnormal: increased number and type of cast significant
- Certain types of casts are always pathologic: RBC, WBC
- Casts are only formed in the **kidney** tubules: not the bladder, not urethra, not ureters

60



Structural Makeup of Casts

- Consists of a uromodulin matrix
- Uromodulin is a glycoprotein formerly called the Tamm-Horsfall protein
- This protein matrix does not react with the protein reagent strip test

61

Cast Formation Enhanced By:

- Acidity of urine
- Increased solute concentration
- Decreased urine flow rate (urine stasis)
- Presence of plasma proteins (albumin, globulins, hemoglobin, myoglobin)

62

Characteristics of Casts

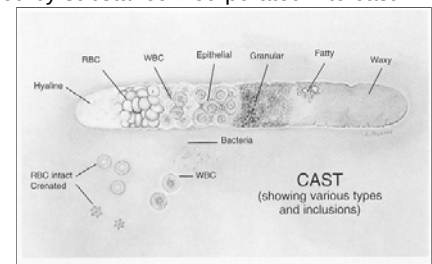
- Cylindrical, cigar shape, parallel sides
- Vary in length and width
- Mucus and fibers can be misidentified as casts



63

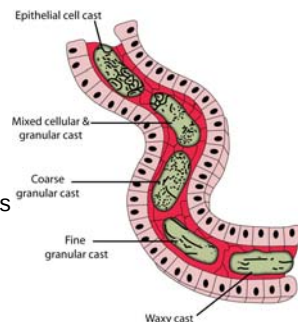
Cast Identification/Classification

- Enumerate using low power objective;
- Identify using high power objective
- Classified by substance incorporated into cast matrix



Cast Identification/Classification

- Youngest cast is the hyaline and cellular, oldest is waxy
- Cast becomes granular and then waxy as the cast ages and substances inside the cast degenerate

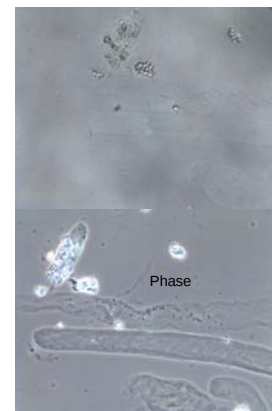


Hyaline Cast

- Low refractive index and homogeneous matrix makes this cast very hard to see using bright field microscopy

Adjust condenser to enhance visualization

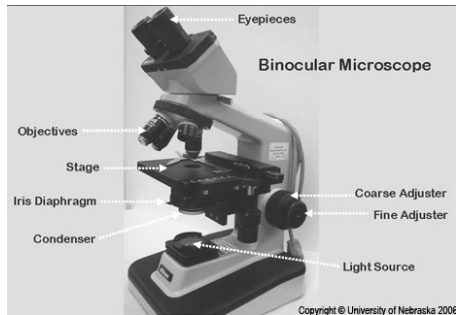
- Phase microscopy used to enhance visualization



6



Lower the condenser to enhance visualization of casts and mucus



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67

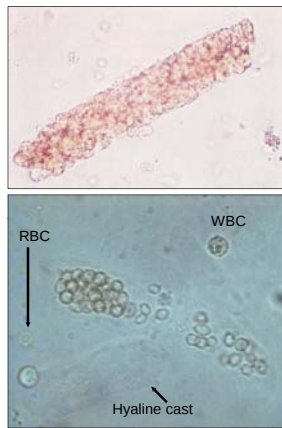
Hyaline Cast

- Most common cast seen in normal individuals
- Normal: 0-2 hyaline casts/lpf
- Increased amounts seen with dehydration, fever, emotional stress, strenuous exercise

68

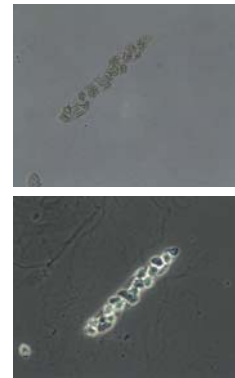
RBC Cast

- RBC inside a hyaline cast
- Cast may appear yellow to reddish-brown color due to degenerating or hemolyzing RBCs
- Significance: **pathologic** condition (not normal)



WBC Cast

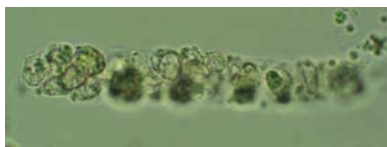
- WBC inside a hyaline cast
- Identify by looking for lobed nucleus
- Significance: **pathologic** condition (not normal)



70

Epithelial Cell Cast

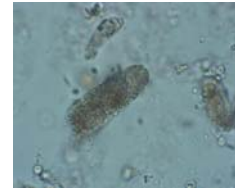
- Always renal tubular epithelial cells in hyaline matrix
- Can be misidentified as WBC cast; look for 1:1 ratio of nucleus to cytoplasm
- Significance: always **pathologic** (never normal)



71

Granular Cast

- Aged cellular cast: fine or coarse granules
- Significance: **pathologic** if increased



72



Waxy Cast

- Highly refractile, homogeneous texture, well defined edges, blunt uneven ends
- May see cracks along the length of the cast
- May appear yellow to gray to colorless
- Significance: **pathologic** (prolonged stasis)

73

Waxy Cast



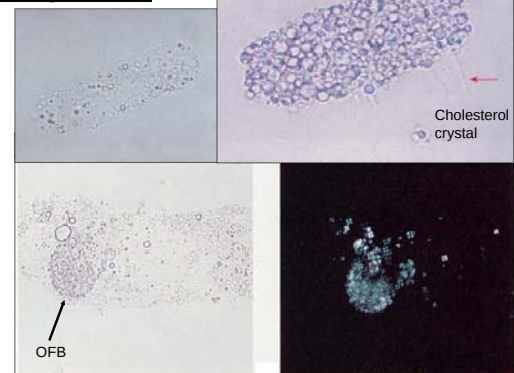
74

Fatty Cast

- Highly refractile due to fat content
- Fat in the form of free fat droplets or oval fat bodies inside the cast
- Identify using polarized microscopy: look for characteristic maltese cross formation
- Significance: **pathologic** finding, often seen in Nephrotic Syndrome

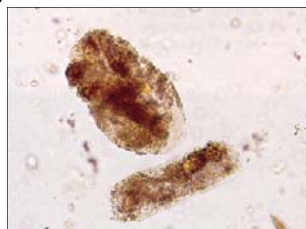
75

Fatty Cast



Broad Cast

- Broad casts are wider than normally seen, since they are formed in the wider collecting ducts
- All types of casts may occur in this wider form
- Significance: **pathologic**



Cast Correlation

- Correlate microscopic evaluation with
 - Physical exam
 - Clarity
 - Chemical exam
 - Protein reagent strip
 - Why ?

78



True / False: Casts are formed in the bladder

True / False: Acid pH and urine stasis are conducive for cast formation

True / False: Casts are enumerated using the high power objective

True / False: Hyaline and RBC casts are not pathologic

79

Crystals

- Not normally found in fresh urine
- If found in fresh urine, pathologic
- Crystals precipitate as urine cools to room temp or when urine is refrigerated
- All clinically significant crystals are found in acid urine

80

Crystal Formation Enhanced By

- Increased concentration of solute in urine
- Urine pH
- Urine stasis
- Temperature

81

Crystal Identification

- Microscopic appearance
- Urine pH

82

Crystal Correlation

- Correlate microscopic evaluation with
 - Physical exam
 - Color
 - Clarity
 - Chemical exam
 - pH

83

Crystals

- Normal acid pH crystals
- Normal alkaline pH crystals
- Pathologic crystals found in acid or neutral urine
- Drug induced crystals

84



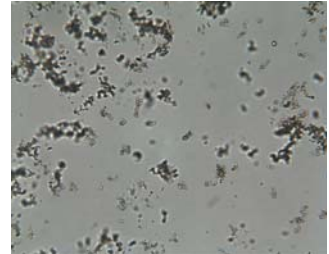
Normal Acid pH Crystals

- Amorphous urates
- Uric acid
- Calcium oxalate

85

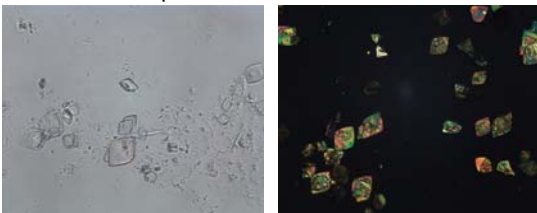
Amorphous Urates

- These crystals have no distinct form and appear as sand-like granules microscopically
- Macroscopically appear as a pink sediment after urine centrifugation
- Acid pH urine
- Often seen after urine is refrigerated

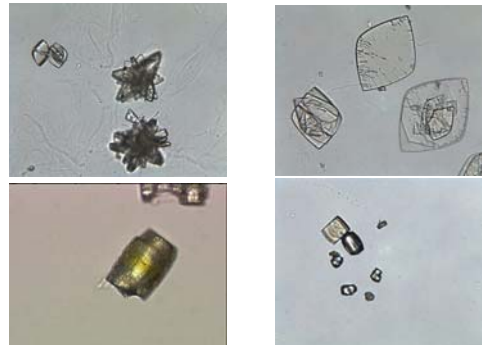


Uric Acid Crystals

- Acid pH urine
- Appear in several forms
- Multicolored when polarized
- Diamond shape most common form



Uric Acid Crystals



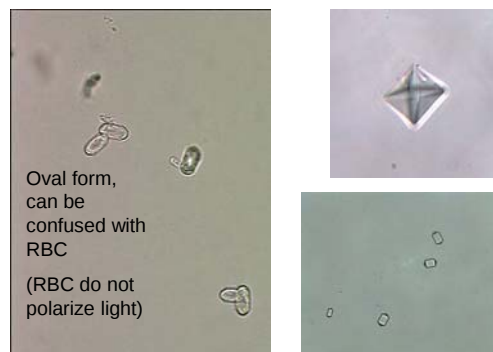
88

Calcium Oxalate Crystals

- Acid pH urine
- Most frequently observed crystal in urine
- Most common form is octahedral shape, often referred to as an 'envelope' shape
- Multicolored when polarized

89

Calcium Oxalate Crystals



90



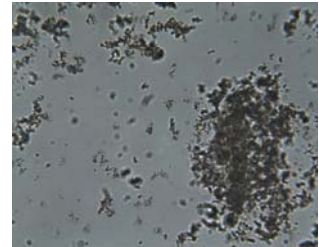
Normal Alkaline pH Crystals

- Amorphous phosphates
- Triple phosphate
- Ammonium biurate

91

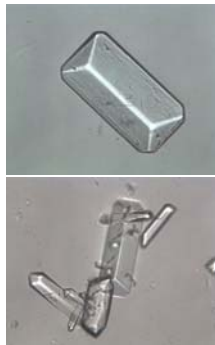
Amorphous Phosphates

- These crystals have no distinct form and appear as sand-like granules microscopically
- Macroscopically appear as a white sediment after urine centrifugation
- Alkaline pH urine
- Often seen after urine is refrigerated



Triple Phosphate Crystals

- Most frequently observed crystal in alkaline urine
- Colorless, 4-6 sided prisms
- Referred to as 'coffin lid crystals'



93

Triple Phosphate vs Calcium Oxalate



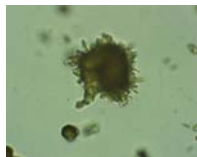
Alkaline pH urine

Acid pH urine

94

Ammonium Biurate Crystals

- Alkaline pH urine
- Yellow spheres with spicules on surface
- Referred to as 'thorny apple crystals'
- Significant when found in fresh urine
- Presence indicates urine is old



95

Pathologic Crystals (acid, neutral pH)

- Cystine
- Tyrosine
- Leucine
- Cholesterol
- Bilirubin

96



Cystine Crystals

- Colorless hexagonal plates
- Do not polarize
- Can be confused with uric acid crystals



97

Cystine vs Uric Acid Crystals



Cystine Crystal
Acid pH urine
Very rare
Do not polarize light



Uric Acid Crystal
Acid pH urine
Frequently seen
Multicolored when polarized⁹⁸

Cholesterol Crystals

- Clear, large, flat, rectangular plates with notched corners
- Multicolored when polarized
- Can be confused with radiographic dye crystals
- Also should see proteinuria and lipiduria

99

Cholesterol Crystals

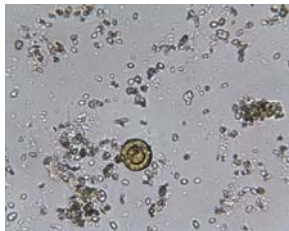


Flat with notched corners

100

Leucine Crystals

- Yellow-brown spheres with concentric circles on surface (tree trunk)
- Can resemble free fat globules



101

Tyrosine Crystals

- Colorless or yellow-brown fine delicate needles

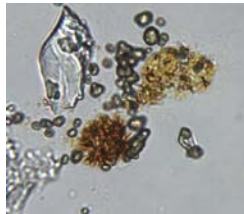


102



Bilirubin Crystals

- Yellow-brown small clusters of needles or granules
- Must confirm with positive icotest



- When present in urine, indicates large amount of bilirubin is present

103

Bilirubin vs Tyrosine Crystals



Bilirubin: thicker needles, blunt ends
Ictotest positive



Tyrosine: fine needles, pointy ends
Ictotest negative

104

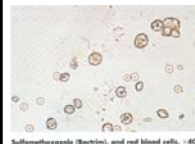
Drug Induced Crystals

- Sulfonamides
- Radiographic dye (contrast media)

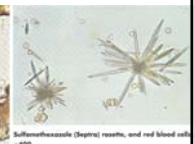
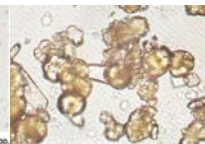
105

Sulfa Crystals

- Form varies dependent upon the type of sulfa drug administered



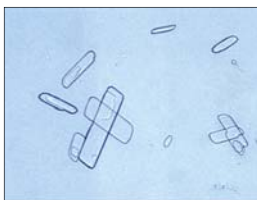
Sulfamethoxazole (Bactrim), and red blood cells, $\times 400$



Sulfamethoxazole (Bactrim) crystals, and red blood cells, $\times 400$

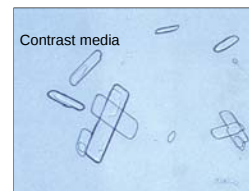
Radiographic Dye Crystals

- Also referred to as Contrast Media
- Colorless long pointed needles, or flat rectangular plates (resemble cholesterol crystals)
- Multicolored when polarized



Contrast Media vs Cholesterol

- Both crystals multicolored when polarized
- Contrast Media: specific gravity > 1.040
- Cholesterol: notched edges





True / False: Crystals are identified by shape and urine pH

True / False: Uric acid crystals are found in acid urine and are shaped like a 'coffin lid'

True / False: Presence of ammonium biurate crystals indicate the urine is old

True / False: Presence of bilirubin crystals indicate severe liver disease

109

Other Microscopic Elements

- Bacteria
- Yeast, mycelial elements (pseudohyphae)
- Fat
- *Trichomonas vaginalis*
- Sperm
- Mucus
- Starch, talc
- Fibers
- Glass, plastic

110

Bacteria

- Most often rod-shaped
- Vary in size
- Must use high power objective
- Presence may indicate
 - UTI
 - Contamination



111

Bacteria

- Correlate with
 - Nitrite reagent strip
- pH often alkaline
 - urea → ammonia
 - ammonia increases the pH

Bacteria DO NOT 'make' the urine alkaline
Bacterial enzyme convert urea to ammonia

112

Yeast and Mycelial Elements

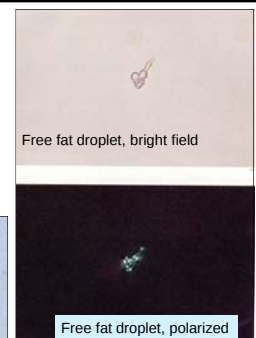
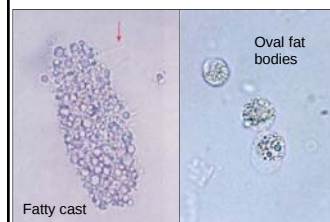
- Budding forms or singly
- Ovoid and more refractile than RBC
- Will not lyse with acetic acid or KOH
- Note pseudohyphae (mycelial elements)



113

Fat

- Highly refractile
- Found in 3 forms:
 - Within fatty cast
 - Within oval fat body
 - Free fat droplet

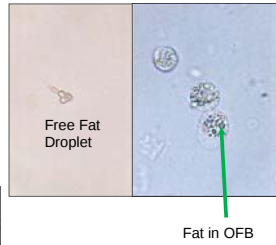


114



Fat vs Starch

- Both polarize light
- Starch has characteristic central dimples



115

Trichomonas vaginalis

- Round to lemon-pear shape
- Undulating membrane and flagella provide movement
- Sexually transmitted
- Similar in size with WBC and RTE
- Can be confused with WBC



116

Spermatozoa

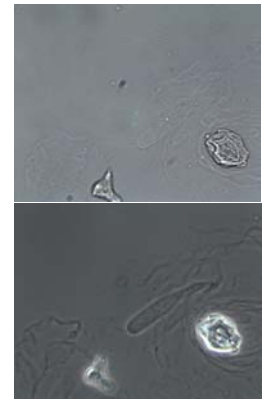
- May be seen in male and female urine
- Usually not clinically significant unless
 - Post vasectomy
 - Rape
 - Child urine



117

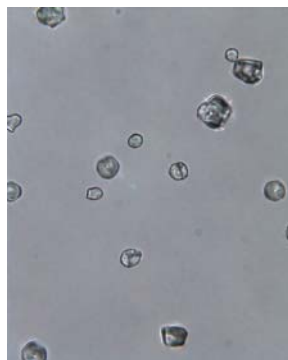
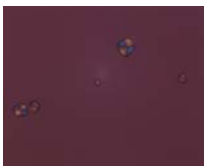
Mucus

- Low refractive index makes it difficult to see
- Wavy, delicate ribbon-like strands or threads
- Can be mistaken for hyaline cast



Starch, talc

- Contaminant
- Varies in size and shape
- Characteristic central dimple



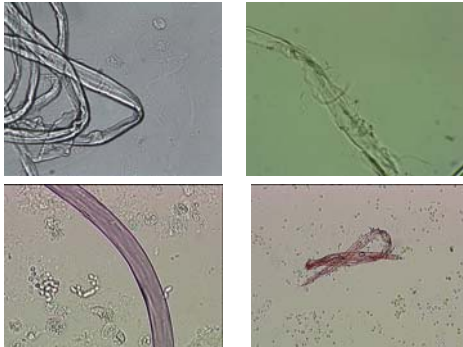
Fibers

- Contaminant
- Large, with distinct edges
- Misidentified as casts





Fibers



121

Glass, Plastic

- Contaminant from
 - Glass cover slips
 - Plastic cover slips
- Misidentified as a crystal

